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PT-Cell Gene Therapy to Eradicate Disseminated Breast Cancers

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PRINCIPAL INVESTIGATOR:

Richard P. Junghans, PhD, MD

CONTRACTING ORGANIZATION:

Roger Williams Medical Center
Providence, RI 02908

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14. ABSTRACT This represents work performed under year 1 of our Breast Cancer IMPACT award. The goals of the award are clinical trials with designer T cells in breast cancer, but also preclinical development work to bring new configurations to the clinic. Following completion of harmonization of the clinical protocol with the HSRRB, patient enrollments are expected to begin in August, 2010. Other laboratory work has been performed to begin to assess the use of additional domains from T cell signaling molecules to improve the characteristics of the designer T cells for patient use. Under US Army support, a new technique has been developed to improve the gene expression for additional signals in the designer T cells for which a patent has been file				
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Table of Contents

	<u>Page</u>
Introduction.....	1
Body.....	1
Key Research Accomplishments.....	4
Reportable Outcomes.....	4
Conclusion.....	4
References.....	5
Appendices.....	

Annual Report

INTRODUCTION

The following are the study Aims. We report progress on three Aims: 1, 3 and 4 (marked by *).

Clinical (existing products):

- *1. To test efficacy of 2nd gen designer T cells in metastatic breast cancer
- 2. To test ancillary procedures for improved persistence and activity of infused designer T cells

Advanced Research & Development:

- *3. To create and test other CIR designs with alternative co-stimulatory domains
- *4. To create and test CIR to avoid need for IL2 supplement in vivo to sustain T cell survival

Clinical (new products):

- 5. To conduct clinical trials with the new generation products.

BODY

- 1. To test efficacy of 2nd gen designer T cells in metastatic breast cancer

This Aim applies a randomization of 12 subjects to –IL2 or +IL2 arms at the maximum tolerated dose (MTD) or maximum practical dose (MPD) of designer T cells under a Phase Ib design. This will test the optimal biologic dose (OBD) in terms of the benefit of IL2 to T cell persistence and activity in vivo. There were three dose levels in the original Phase Ia: 10^9 , 10^{10} and 10^{11} T cells. We are screening our final patient on the 10^{10} dose level. To date, all patients have had good tolerance of the designer T cells and we anticipate that this final patient will display the same pattern. To date, one patient has had a minor response to treatment, with shrinkage of brain and lung mets, but with resurgence of disease in subsequent months. This is a situation in which we postulate that addition of IL2 will allow a more prolonged and deeper response. A second patient has had stable disease without other effective treatment for 12+ months.

During the past year, we completed all regulatory steps prior to the patient enrollments. This involved numerous interactions with the US Army HSRRB to harmonize the protocol and consent form with the DOD standard. Further, we obtained permission from the FDA and IRB to merge the final step of the Phase Ia with the Phase Ib to treat the first patients at the 10^{11} T cell dose at the same time as the IL2 randomization. This saves time and resources, allowing start of the breast cancer Phase Ib portion of study sooner without compromising patient safety. The final IRB review of the harmonization was conducted this past month with some minor comments, but full approval anticipated with IRB signature on June 7, 2010.

During this period, we conserved resources for the clinical trial that were not expended in the first year while awaiting harmonization with the DOD HSRRB and completion of the Phase Ia

dose escalation. This carry-over amounts to approximately \$250,000.

We anticipate enrollment of the first patient on the Phase Ib study under this research plan during the month of August, to follow with rapid accrual over the ensuing year. In anticipation of this date, the PI met with Dr Susan Love at the AACR meeting in April 2010, whose Million Women foundation will support our recruitment efforts with emailings and postings. This will materially improve recruitments and will supplement awareness efforts of Ms Marlene McCarthy of the RI Breast Cancer Coalition, who is on the study as patient advocate.

Finally, we have prepared in collaboration with the National Gene Vector Lab a new stock of retroviral supernatant that will serve the project. This is 20 liters in volume and has 50% greater titer than our prior production run.

*3. To create and test other CIR designs with alternative co-stimulatory domains

We proposed a plan to perform high through-put testing of second co-stimulatory molecules in the format of Ig-CD28-X-zeta, in which Ig represents the MN14 antibody recognition domain, and X is the second co-stimulatory molecule. (CD28 is the first.) Constructs have been prepared for all of the following co-stimulatory molecules. These include X = HVEM, 4-1BB, ICOS, OX40, and CD27. This work was performed by Dr Bais.

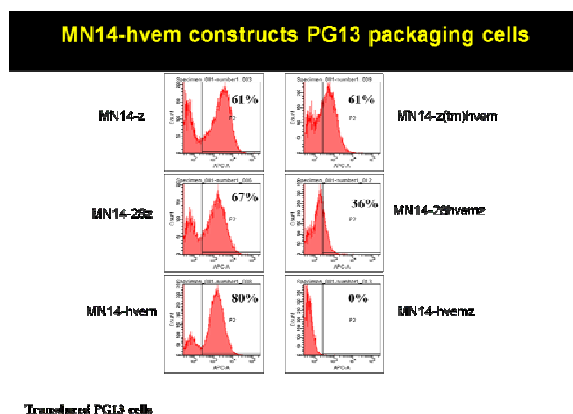
MN14-X
MN14-z(tm)-X
MN14-28-X-z
MN14-X-z

At this point, we can report early data on the HVEM constructs. Controls are MN14-zeta (1st generation) and MN14-CD28-zeta (2nd generation). These both express well in PG13 cells, our vector producer cell line. With HVEM, we characterized the following.

MN14-HVEM
MN14-z(tm)-HVEM
MN14-28-HVEM-z
MN14-HVEM-z

Of these, the MN14-HVEM expressed the best, followed by MN14-z(tm)-HVEM (using the zeta TM domain), then MN14-28-HVEM-z, and non-expressing MN14-HVEM-z.

The desired configuration with 3 signals in the single construct is thus shown to be problematic for expression. The origin of this problem is not obvious, but it suggests that this construct cannot be effectively tested in our T cells.



An alternative will be pursued. This will be to co-express MN14-28-z and MN14-HVEM in the same vector, separated by a 2A sequence for two-gene expression. However, it was previously shown in this lab that repeated sequences with the MN14 segment leads to recombination-deletion to leave one gene rather than two. That is, a sequence of A-b ... A'-c will recombine during retroviral replication to generate a single sequence of A-c (Q Ma and RPJ, unpublished results).

Accordingly, to make this two-gene construct possible, Dr Bais undertook a mutagenesis procedure that wobbles the bases for every amino acid possible in one of the repeated elements. This was performed in a model system and shown to suppress the recombination-deletion, and will be published in the near future as a work product of this award. The procedure will now be applied to the HVEM construct.

Further characterization of other 3 signal constructs will be performed to determine if these likewise require a two-gene vector for expression.

*4. To create and test CIR to avoid need for IL2 supplement in vivo to sustain T cell survival

There are three parts to this Aim:

- 4.1) to examine Signal 3 (LFA1 and CD2) for their capability to yield sustained IL2 on re-stimulation with antigen,
- 4.2) to express IL2 constitutively from a promoter within the transferred transgene, and
- 4.3) to express anti-apoptotic genes that make cells resistant to IL2 withdrawal.

We have made progress on subaim #4.1, and are presently configuring a manuscript for publication. We previously had shown that IL2 secretion with Signal 1+2 was abundant, but that IL2 release was lost on successive antigen restimulations [1]. We recently published that IL2 was essential for in vivo responses with 2nd generation designer T cells in animal models, despite the high IL2 secretion on antigen contact [2]. Our hope had been that Signal 3 would overcome the block to IL2 secretion on restimulations to help the T cells replicate in vivo without exogenous IL2 stimulation. [NB: the co-stimulatory molecules of Aim 3 are all considered Signal 2, although in tandem with zeta and CD28 will provide three signals. We reference CD2 and LFA1 as Signal 3 being qualitatively different, based on data of Sprent and Schlom. See grant proposal for references.]

In our experiments, we stimulated resting and activated T cells with Signal 1 or Signal 1+2 and assessed the impact of adding Signal 3 on IL2 and gamma interferon secretion. In all cases, Signal 3 provided a major increment in cytokine secretions. On restimulation, however, we found improved IL2 persistence, but it was still insufficient IL2 production to be self-sustaining. Thus, the hypothesis of sufficiency for IL2 production with three signals was falsified. This shifts the hope for avoiding systemic IL2 costs and toxicities to the subsequent subaims.

We also created 3-signal designer T cells, of the format Ig-CD28-X-zeta, in which X is Signal 3. Because of problems with expression, we were not able to obtain a CD2 based construct. Instead, our focus was on the separate analysis of the LFA1a and LFA1b chains. Our tests showed that the IL2 secretion signal was primarily from the LFA1 beta chain, and not the alpha chain. However, both provided co-stimulation signals that enhanced proliferation of the designer T cells. These data were previously unknown, and speak to the outside-in signaling of the LFA1 that is primarily thought of as an adhesion molecule.

KEY RESEARCH ACCOMPLISHMENTS

1. Completion of harmonization of clinical trial protocol and consent with HSRRB.
2. Finalized protocol resubmitted to RWMC IRB with approval.
3. Coordination with Dr Susan Love of the Million Women foundation for patient recruitments. This will complement efforts of Ms Marlene McCarthy of the RI Breast Cancer Coalition.
4. Retroviral vector constructs prepared for multiple costimulatory molecules in 3-signal single gene format.
5. Testing of expression of the HVEM constructs, showing problem for single chain format.
6. Validation of mutagenesis methods for allowing retrovirus with two gene expression with repeated MN14 domains.
7. Retroviral vector constructs prepared that incorporate Signal 3.
8. Demonstration of improved IL2 and gIFN secretion with Signal 3.
9. Definition of LFA1 beta chain as that conferring IL2 production.
10. Falsification of hypothesis of Signal 3 allowing sustained IL2 secretion on restimulation.
11. Definition of improved proliferation with the Signal 3-containing designer T cells.

REPORTABLE OUTCOMES

1. Patent filing:

Bais A, Yang W, Junghans RP. 2010. Viral vectors encoding multiple highly homologous polypeptide domains.

CONCLUSION

Progress is being made on the Aims of the proposal. The Phase Ib study is set to initiate by August 2010, with a rapid accrual planned. The laboratory research for more advanced generation clinical products is at an early stage but proceeding appropriately.

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- ² Lo ASY, Ma Q, Liu DL, Junghans RP. Anti-GD3 chimeric sFv-CD28/T cell receptor zeta designer T cells for treatment of metastatic melanoma and other neuroectodermal tumors. Clin Cancer Res 2010;16:2769-80.